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Asymmetric Division: AGS Proteins Position the Spindle

Dispatch

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Many cells divide asymmetrically by shifting their division machinery toward a specific region of the cell cortex, but little is known about how this occurs. Three recent papers have implicated activators of heterotrimeric G protein signaling in this process in *Caenorhabditis elegans*.

There was a time, not long ago, when finding a role for heterotrimeric G proteins in a biological process was taken as a sign that cells were signaling to other cells. The functions of heterotrimeric G proteins depended on cell–cell signaling: cells sent signals that could activate serpentine receptors on the surfaces of other cells, and the job of heterotrimeric G proteins was to relay these signals from the serpentine receptors to targets inside the cell [1].

Then, in 1996, heterotrimeric G proteins were implicated in orienting the mitotic spindle in the one-cell stage embryo of the nematode worm *Caenorhabditis elegans* [2]. This was a perplexing finding, as the one-cell stage embryo — the zygote — has essentially no neighbor from which to receive signals. The *C. elegans* zygote is, in fact, shielded from communication, encased in envelopes that are impermeable to proteins and even to many small, cell-permeant molecules [3]. The only other occupant of these envelopes is the tiny remnant of meiosis, the polar body, which appears to play no essential role in development.

What is a protein complex involved in cell–cell signaling doing to position the spindle in such a reclusive cell? A possible solution to this paradox came when mammalian proteins were identified that can activate heterotrimeric G protein signaling independently of cell surface receptors [4,5]. These were named the activators of G protein signaling or AGS proteins. Three new papers [6–8] have now reported evidence that a pair of *C. elegans* AGS proteins, called GPR-1 and GPR-2, can regulate heterotrimeric G protein signaling to position the mitotic spindle in the zygote. Together with the finding that an AGS3 homolog positions spindles in *Drosophila* (see [9] for review), the results suggest that receptor-independent activation of heterotrimeric G protein subunits may be part of a conserved machinery for positioning mitotic spindles.

The first division of the *C. elegans* zygote produces two cells of differing sizes, because the mitotic spindle shifts position during mitosis (Figure 1). The spindle forms in the center of the cell, but just before the chromosomes segregate, the spindle moves toward the posterior end of the cell [10]. After it moves, cytokinesis occurs near the center of the mitotic spindle, resulting in division of the cell posterior of its center. Some asymmetries can be seen in the mitotic spindle itself as the spindle arrives at the posterior: the posterior centrosome rocks back and forth, and it also flattens [11].

The three groups that converged on the *C. elegans* GPR-1/2 proteins met from three different paths. One group was looking for homologs of the mammalian AGS proteins [6]; another was looking for proteins that interact with the spindle positioning protein LIN-5 [7]; and the third was looking for genes required for normal cell divisions in a functional genomic screen [8]. All three groups found that using RNA interference (RNAi) to reduce the function of GPR-1/2 converted the normally asymmetric cell division of the *C. elegans* zygote into a symmetric cell division, a phenotype that had been seen previously in Gα loss-of-function backgrounds [12,13].

The first heterotrimeric G protein subunit implicated in spindle positioning was a *C. elegans* Gβ subunit [2], but Gβ regulates not the posterior shift of the spindle, but an earlier process which determines the angle at which duplicated centrosomes migrate away from each other. It is Gα that controls the shift of the spindle toward the posterior of the cell [13]. Elimination of Gα also prevents asymmetries in the mitotic spindle, such as posterior rocking and flattening, from appearing [12,13], and elimination of the newly identified AGS proteins, GPR-1/2, produced similar results [6–8]. GPR-1/2 and Gα do not appear to initiate cell polarization, as they do not affect the asymmetric distributions of the PAR proteins, components of a conserved machinery for polarizing cells [6–8,11]. Instead, they appear to

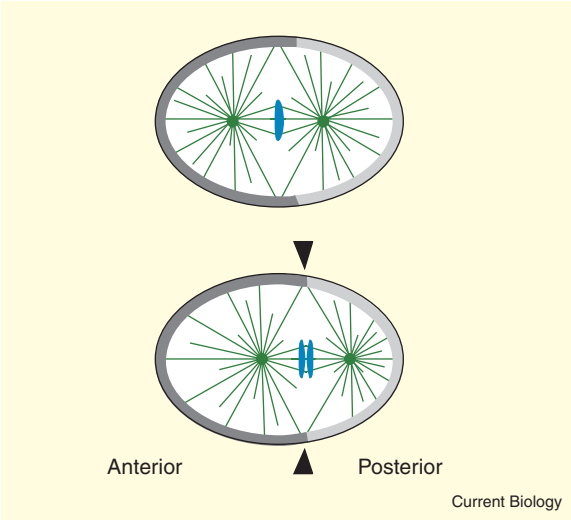

Figure 1. The mitotic spindle in the *C. elegans* zygote before (top) and after (bottom) the posterior shift in spindle position.

Microtubules (green), chromatin (blue) and anterior and posterior PAR proteins (dark and light gray, respectively) are shown. Arrowheads mark where cytokinesis will occur.

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function downstream of this machinery to position the spindle [6,8].

GPR-1/2 can interact directly with Gα in its GDP-bound form and appear to function as guanine nucleotide dissociation inhibitors (GDIs), as shown for AGS proteins in *Drosophila* and mammals [6–8,14,15]. GDIs normally function in opposition to proteins that promote guanine nucleotide exchange (GEFs), but loss of a predicted *C. elegans* GEF results in a phenotype similar to that resulting from loss of Gα and GPR-1/2 [12,16], suggesting that they are not working in opposition in this system. Whether the GDP-bound or the GTP-bound form of Gα is the effector of this signaling pathway is not yet clear.

C. elegans GPR-1/2 are present in a spotty distribution throughout the cytoplasm, and they are enriched near centrosomes and at the cell cortex [6–8]. The cortical enrichment is especially interesting. One group [7] reported that GPR-1/2 appeared to be symmetrically enriched throughout the cell cortex, while the other two groups [6,8] reported that a detectable asymmetry exists for a short time: during mitosis, GPR-1/2 appear somewhat more concentrated in the posterior, the end toward which the mitotic spindle is moving [6,8]. This would suggest that Gα, which is enriched in the cell cortex but has not been reported as asymmetrically distributed [12,13], may be differentially activated on the two sides of the cell.

How does Gα signaling position the spindle? Spindle-cutting experiments in *C. elegans* have revealed that the anaphase mitotic spindle is under tension, as it is pulled from both sides, and that this pulling is stronger on the posterior side. This has led to the hypothesis that the forces that separate chromosomes are asymmetrically applied to the spindle by microtubule interactions with differing regions of the cell cortex [17]. The machinery that initially polarizes the cell, the PAR proteins, governs the strength of the pulling force on each side of the cell, as loss of an anterior PAR protein results in posterior-like forces on both sides of the spindle, and *vice versa* [17]. Loss of Gα or GPR-1/2 produces a different result — a nearly complete loss of any pulling force, on either side of the spindle [6,8], and pulling forces that normally exist after spindle positioning are also lost [18].

These results suggest that signaling from Gα activates pulling forces, possibly by activating a microtubule pulling motor that is cortically enriched in the zygote, such as dynein [19]. A recent experiment aimed at estimating the number of active pulling motors just after the spindle has shifted produced a surprisingly low estimate — that there are only a few dozen active pulling motors per cell [18]. If true, this would mean that only a small proportion of the hundreds of microtubules that reach the cell cortex are pulled at any one time, and that most microtubules might occlude movement of the spindle, as they reach the cortex but do not engage active pulling motors. Direct observations of microtubule stability at the cell cortex have revealed that microtubules are less stable at the posterior cell cortex; the removal of occluding microtubules in the posterior might make it easier for the spindle to shift toward this end [20]. Gα is required to produce this asymmetry in microtubule stability [20]. Whether Gα shifts the mitotic spindle solely by activating pulling forces, or also through this asymmetric modulation of microtubule stability, remains to be determined.

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